Applicants: Jonathan A. Bard, et al.
U.S. Serial No. 10/006,343
Filing Date: December 3, 2001
Title of the Invention: DNA ENCODING GALANIN
GALR3 RECEPTORS AND USES THEREOF
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FIGURE 1

```
   1 AGCTCCAGCCTAGGCGTTCTACCTGGAAGAATGCAGGGGCCCAGTACCTAGGACTGAGGA   60
  61 AGATGGCTGACATCCAGAACATTTCGCTGGACAGCCCAGGGAGCGGTAGGGCTGTGGCAG  120
 121 TGCCTGTGATCTTTGCCCTCATCTTCCTGTTGGGCATGGGCAATGGGCTGGTGTTGG     180
 181 CTGTGCTACTGCAGCCTGCCCAAGTGCCTGGCAGGAGCCAAGCAGTACCACAGATCTCT   240
 241 TCATCCTCAACTTGGCCGTGGCCGACCTTTGCTTCATCCTGTGCCGTGCCCCTTCCAGG   300
 301 CAGCCATCTACACACTGGATGCCTGGCTCTTTGGGCTTTGCGTTGCAAGACGGTACATC   360
 361 TGCTCATCTACCTCACCATGTATGCCACTGCGCACCCACTGCGCTTCACCCTGGCCGTCCCTGGACA   420
 421 GGTACCTGGCTGTGCGGCACCCACTTCGCTGCTGTGGGGCCCGCCCTACCTAAGCT     480
 481 GCGCCGCGTGGGGCTCGTGCGGCTGCTGTGCTGTGGAGGACGCGC            540
 541 ATTACGGCACGGTGCGCGCTACGGCGCCGCCACCTTCGCCGCGGGCTACCTGCGGCCGTGG   600
 601 GGCGGGCGCGCGTGGACCGTGACGCCGCCCGTATGGGCCGTCCCGGCGGGGCG         660
 661 TGAGCCTGGCCTACGGACGCACGCTATGTTTCCTATGGCGCCGTGGCCGGCCATGCTGGCAG   720
 721 CGGGGCAGCAGAGGCGCGCAGACGGGCGACCGGCCGCCATGCTGGCAG          780
 781 TGGCCGCGCTCTACGCGCTTTGCTGCCCGGCCCACCACGCCGCTTCGCTTCTGGT        840
 841 ACGGCCGCGTTCGCCTTCAGCCCGGCCCTACGCCCTGGCCTCGCACTGCCCTCG        900
 901 CCTACGCCAACTCCTGCCTTAACCCGTCGTCTACTGCGCCTGCCCACTTCCGCG        960
 961 CGCGCTTCCGCCCTGTGGCCCTGCCCTGCCCGGTCTTCGGGCCGGTTATCCCGGCGACG   1020
1021 ATCGAGCCCCTCCGTCGTCCAGCCGTGTCCAGCCGGTATGCGAGCCCAGAGGGGACG     1080
1081 CCAGGCCTCGTGGTTGGAGTATGGAGCCCAGGGGATGCTCTGCGTGGTGGAGAGA      1140
1141 CTAGACTAAACCCTGTCCCCAGGGACCTCAATAACCCTGCCCGCTTGGACTCTGACGTC   1200
1201 TGTCAGAATGCCACCAAGGAACATCTAGGGAACGGCCAGTCTCCGCCAGGCTCCACCAAAAA   1260
1261 GCAGAAGCAAAGTTGCAGGG                                          1280
```

FIGURE 2

Amino acid sequence (single-letter code), with residue position numbers:

```
  1  M A D I Q N H S L D   S P G S V G A V A V   20
 21  P V H F A L L F L L   G M V G S N G L L A   40
 41  V L L Q P G P S A W   Q E P S S T D F L F   60
 61  H L N L A V A D L C   F H L C C V P F Q A   80
 81  A H Y T L D A W L F   G A F V C K T V H L  100
101  L H Y L T M Y A S S   F T L A A V S L D R  120
121  Y L A V R H P L R S   R A L R T P R N A R  140
141  A A V G L V W L L A   A L F S A P Y L S Y  160
161  Y G T V R Y G A L E   L C V P A W E D A R  180
181  R R A L D V A T F A   A G Y L L P V A V V  200
201  S L A Y G R T L C F   L W A A V G P A G A  220
221  A A A E A R R R A T   G R A G R A M L A V  240
241  A A L Y A L C W G P   H H R L I L C F W Y  260
261  G R F A F S P A T Y   A C L L A S H C L A  280
281  Y A N S C L N P L V   Y S H A S R H F R A  300
301  R F R R L W P C G R   R R P R H H P R A H  320
321  R A L R R V Q P A S   S G A A G Y G G D A  340
341  R P R G W S M E P R   G D L R G G G E T    360
361  R L T L S P R G P Q                        370
```

FIGURE 3

```
   1  CACTCAGGCGATGACTTTGGCTCTGCTCCCCTCCCATCTCCCACGAGCTTCCCAGCCC       60
  61  AGAACACCTGGCCAGACCCAGTCGGGGGAGTTAGATCCCGGGTCAAGCAACCAGAACT      120
 121  GGGGCTCTTGCCTGAGGATTCCAGCTTCTTCCCCAGTGCCCGTCTGATGGGGAGATG      180
 181  GCTGATGCCCAGAACATTTCACTGGACAGCCCAGGAGTGTGGGGGCCGTGGCAGTGCCT     240
 241  GTGGTCTTTGCCCTAATCTTCCTGCTGGGGCACAGTGGGCAATGGGCAGTGGCTGGCAGTG   300
 301  CTCCTGCAGCCTGGCCCGAGTGCCCTGGCAGGAGCCTGGCAGCCACGACCTGTTCATC      360
 361  CTCAACCTGGCCGGTGGCTGACCTCTGCTTCATCCTGCTGCCCTTCCAGGCCACC        420
 421  ATCTACACGGCTGGATGCCTGGCTCTTTGGGCCCTCGTTTACGCTTTACGCTTTACGGTAC   480
 481  ATCTACCTCACCATGTACGCCAGCAGCTTTTACGCTGCTGCTGTCCGTGGACAGGTAC      540
 541  CTGGCCCGTGGCACCCGGTGCGGTGCCACCCGCTTCGCGCACGCCCGCGCC            600
 601  GCAGTGGGGCTGGTGTGGGCTGCTGGCGCGGCTCTTCTTCGCGGCTCCTACCTCAGCTACTAC 660
 661  GGCACCGTGCGCTACGGCGCTGGAGCTCTGCGCGTGCCCGTGGGGAGGACGCGCCGC      720
 721  CGGCGCCCTGACGTGGCCACGTGCCACCTTCGCGCGTTCCTGTGGGCCGGGCGCGGCG     780
 781  CTGGCCTACGGGGCGCACGCTGGCCCGTGTCCTGTGGGCCGGCGCGCGGCGCGGCG      840
 841  GCGGCCGGAGGCGGCGGCGGAGGGCGACGGGCTCCCGGGGCGCCATGCTGGCCGGTGGCC   900
 901  GCGCTCTACGGCCCTGCTGGGGCGCCACCGCGCCCTCATCCTGTGCTTCTGCTTCTGGTACGGC  960
 961  CGCTTCGCCTTCAGCCCGGCCACGCTACGCGCCCTGGCCCTCACACTGCCTGGCCTAC   1020
 021  GCCAACTCCCTGCCTCAACCCCGTCGTTCACCCGCGTCTACGCGCCCACTTCCGCGCGCGC   1080
 081  TTCCCGCCCTGTGCTGCCCCGTTGCCGACCGCCGCCGACGCCCGCCCGCCCTTGCGT    1140
 141  CGCGTCCCGCCCGCCGTCCTCGCGCCTTCGCGGCCCACCCGTGCCCGGAGACGCCCTAGCGGG  1200
 201  AGGCTGCTGGCTGGTGGCGCGACCGGAATAAACCCTGCGCCCGTCCACGGCGGACCCCGGGA  1260
 261  GAGGCCCGAGGACCGGAGACGCCCTGGACTCCGCCCTGTGTCCGTCTGTC            1320
 321  TCACTCCCGTTCTCCGAAGGCGGGGACGCGCCACCGGGCCAGGGGATGGGGCAATGCCACGAGC 1380
 381  TCTCTGAGGGGCCGTTGAGTGGAGCCGACTTGTCCCCGC                        1417
```

FIGURE 4

Pos.	Sequence
1	H S A M T L L L L S P P P S P T S F Q P
21	R T P G Q T Q V G G V R S R R Q A T R T
41	G G S C L R H P A S L P R C G S D G E M
61	A D A Q N H S L D G P G S V P A V A V P
81	V V Q A L H F L L Q T V G N G L V L A V
101	L L F P G P S A W F E P G S T T D L F H
121	L N Q A A S D L C G H L C C V P F Q A T
141	H Y L L V A W L F F A L V A K A V H L L
161	H Y T T D Y A S S R T L A T V S N D R Y
181	L A L R M P L R A A A L R A P R L A R A
201	A V V L H W L L E L L F S A P Y D S Y Y
221	G T G R V G A L A A C V P A W E A A R R
241	R A V D Y A T F F L G Y L L L V A V V S
261	L A L G V T L R T G W A A V P P L G A A
281	A A Y A R R R A P H R A G R G M F A V A
301	A L E A R C W G Y A H R L I A C C W Y G
321	A F Y F L P A T V Y C L L A L H H L A Y
341	R N A C S N P L R R A R A S S H F R A R
361	A R S L L P C G P P R C H R R R R A L R
381	F V R P W S S G G P G P P G A A R P S G
401	R L V A A G G Q ... E ... R ... R ...
421	R A L R G G G ... E

(Column position markers: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440)

FIGURE 5A

```
hGALR3  . . . . . .  M A D A Q N I S L D S P G . . .        13
rGALR3  . . . . . .  M A D I Q N I S L D S P G . . .        13
rGALR1  M E L A P V  N L S E G N G S D P E P P A E P R P L  25

hGALR3  . . . S V G  A V P V V F A L I F L L G T V G N      35
rGALR3  . . . S V G  A V P V I F A L I F L L G M V G N      35
rGALR1  F G I G V E  N F I T L V V F G L I F A M G V L G N  50
                                              I

hGALR3  G L V L A V  L L Q P G P S A W Q E P G S T T D L F  60
rGALR3  G L V L A V  L L Q P G P S A W Q E P S S T T D L F  60
rGALR1  S L V I T V  L A R S K P G . . K P R S T T N L F    72
                                              II

hGALR3  I L N L A V  A D L C F I L C C V P F Q A T I Y T L  85
rGALR3  I L N L A V  A D L C F I L C C V P F Q A A I Y T L  85
rGALR1  I L N L S I  A D L A Y L L F C I P F Q A T V Y A L  97
```

FIGURE 5B

```
                                        III
hGALR3   D A W L F G A L V C K A V H L L I Y L T M Y A S S   110
rGALR3   D A W L F G A F V C K T V H L L I Y L T M Y A S S   110
rGALR1   P T W V L G A F I C K F I H Y F F T V S M L V S I   122

hGALR3   F T L A A V S V D R Y L A V R H P L R S R A L R T   135
rGALR3   F T L A A V S L D R Y L A V R H P L R S R A L R T   135
rGALR1   F T L A A M S V D R Y V A I V H S R R S S S L R V   147

                 IV
hGALR3   P R N A R A A V G L V W L L A A L F S A P Y L S Y   160
rGALR3   P R N A R A A V G L V W L L A A L F S A P Y L S Y   160
rGALR1   S R N A L L G V G F I W A L S I A M A S P . V A Y   171

hGALR3   Y G T V . . R Y G A L E L C V P A W . E D A R R R   182
rGALR3   Y G T V . . R Y G A L E L C V P A W . E D A R R R   182
rGALR1   Y Q R L F H R D S N Q T F C W E H W P N Q L H K K   196
```

FIGURE 5C

```
                         V
hGALR3   A L D V A T F A A G Y L L P V A V V S L A Y G R T   207
rGALR3   A L D V A T F A A G Y L L P V A V V S L A Y G R T   207
rGALR1   A Y V V C T F V F G Y L L P L L I C F C Y A K V     221

hGALR3   L R F L W A A V G P A G A A A A E A R R A T G R     232
rGALR3   L C F L W A A V G P A G A A A A E A R R A T G R     232
rGALR1   L N H L H K K L K N M S K K S E A S K K · · K       242

                                          VI
hGALR3   A G R A M L A V A A L Y A L C W G P H H A L I L C   257
rGALR3   A G R A M L A V A A L Y A L C W G P H H A L I L C   257
rGALR1   T A Q T V L V V V V F G I S W L P H H V I H L W     267

                                     VII
hGALR3   F W Y G R F A F S P A T Y A C R L A S H C L A Y A   282
rGALR3   F W Y G R F A F S P A T Y A C R L A S H C L A Y A   282
rGALR1   A E F G A F P L T P A S F F F R I T A H C L A Y S   292
```

FIGURE 5D

```
hGALR3   N S C L N P L V V Y A L A S R H F R A R F R R L W P   307
rGALR3   N S C L N P L V V Y S L A S R H F R A R F R R L W P   307
rGALR1   N S S V N P I I Y A F L S E N F R K A Y K Q V F K     317

hGALR3   C G R R R · R H R A R R A L R R V R P A S S G         329
rGALR3   C G R R R H R H H H R A H R A L R R V Q P A S S G     332
rGALR1   C R V C N E S P H G D A K E K N R I D T P P S T N     342

hGALR3   P P G C P G D A R P S G R L L A G G G Q G P E P R     354
rGALR3   P A G Y P G D A R P R G W S M · · · · E P R           350
rGALR1   C T H V · · · · · · · · · · · · ·                     346

hGALR3   E G P V H G G E · · A A R G P E                       368
rGALR3   G D A L R G G G E T R L T L S P R G P Q               370
```

FIGURE 6A

FIGURE 6B

FIGURE 7

Normalized Response

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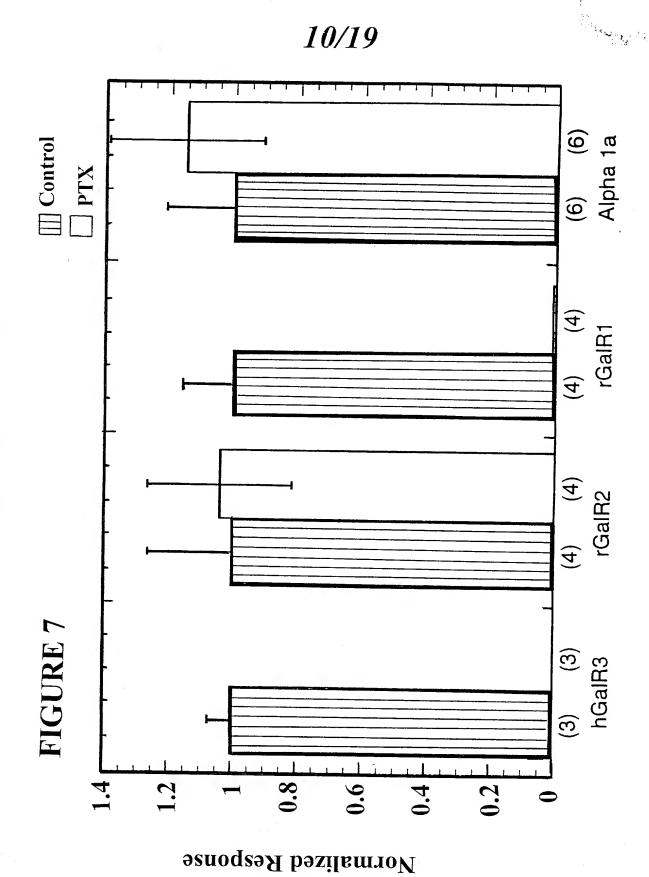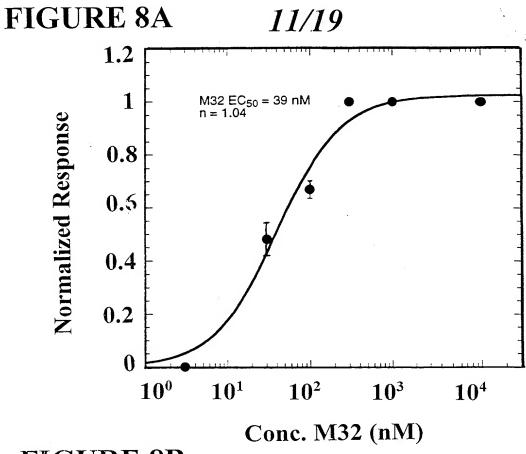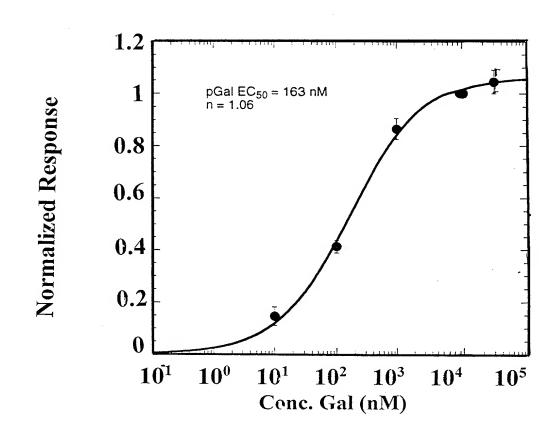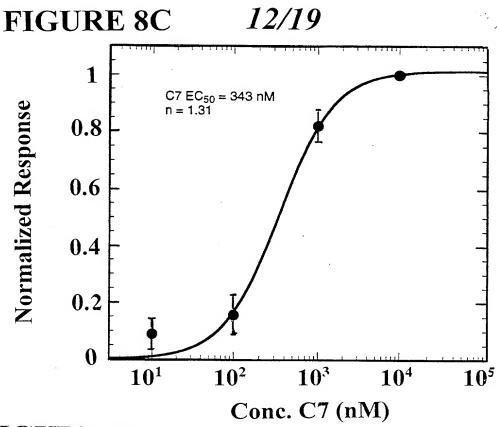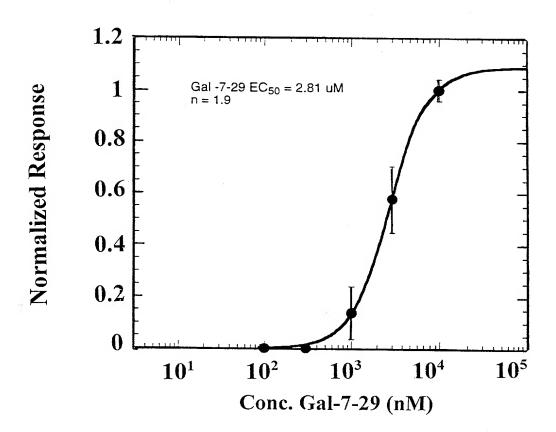GALR3 RECEPTORS AND USES THEREOF
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FIGURE 8A

M32 EC$_{50}$ = 39 nM
n = 1.04

Conc. M32 (nM)

FIGURE 8B
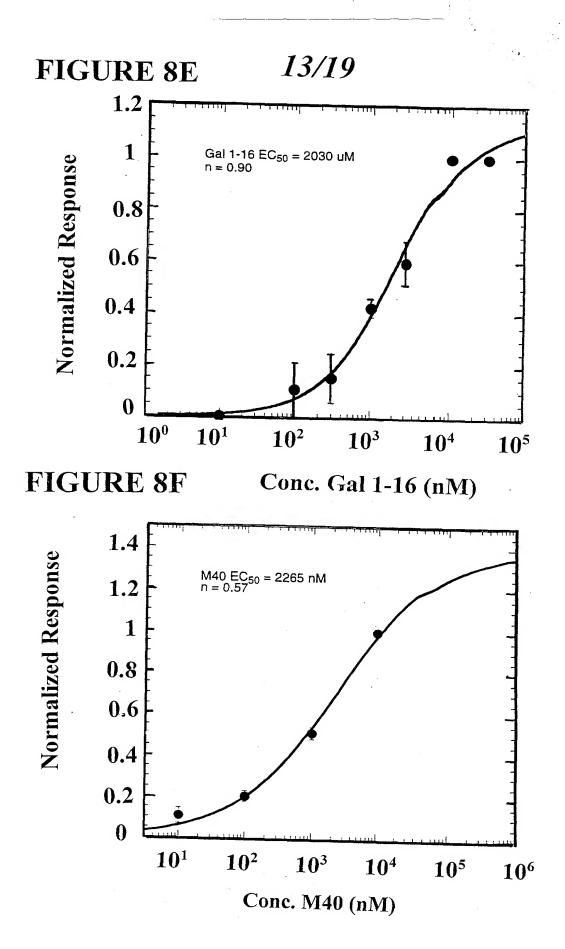

pGal EC$_{50}$ = 163 nM
n = 1.06

Conc. Gal (nM)

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FIGURE 8C   *12/19*

C7 EC_{50} = 343 nM
n = 1.31

Normalized Response

Conc. C7 (nM)

FIGURE 8D

Gal -7-29 EC_{50} = 2.81 uM
n = 1.9

Normalized Response

Conc. Gal-7-29 (nM)

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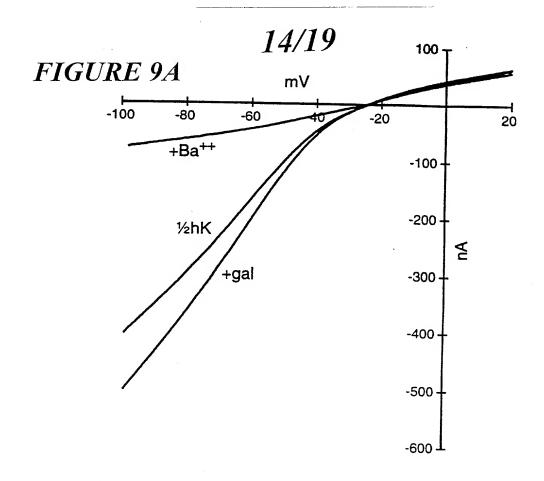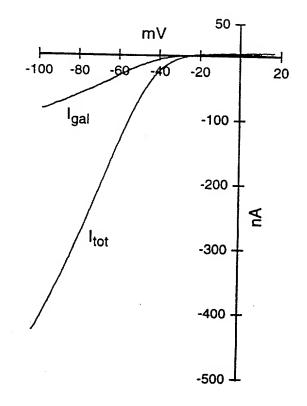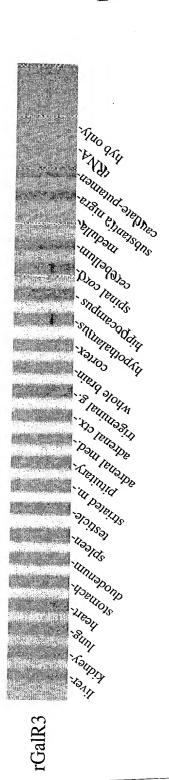GALR3 RECEPTORS AND USES THEREOF
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FIGURE 8E   *13/19*

Gal 1-16 EC$_{50}$ = 2030 uM
n = 0.90

Conc. Gal 1-16 (nM)

FIGURE 8F

M40 EC$_{50}$ = 2265 nM
n = 0.57

Conc. M40 (nM)

FIGURE 9A

FIGURE 9B

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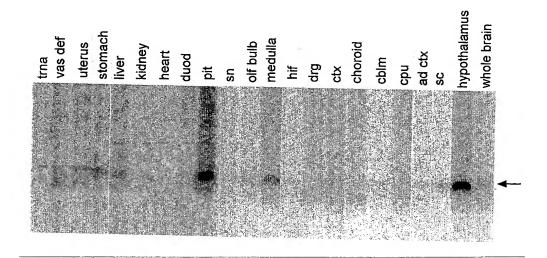GALR3 RECEPTORS AND USES THEREOF
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FIGURE 10

rGalR3

liver-
kidney-
lung-
heart-
stomach-
duodenum-
spleen-
testicle-
striated m.-
pituitary-
adrenal med.-
adrenal ctx.-
trigeminal g.-
whole brain-
cortex-
hypothalamus-
hippocampus -
spinal cord-
cerebellum-
medulla-
substantia nigra-
caudate-putamen-
(RNA-
hyb only)-

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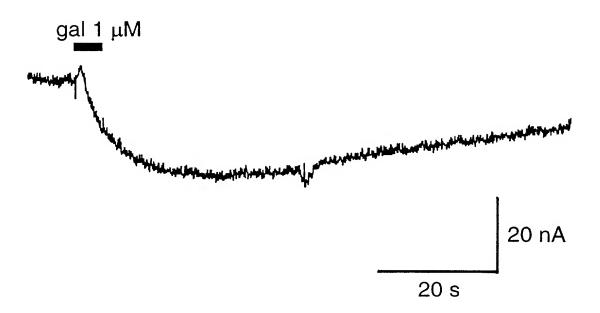Sheet 16 of 19

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FIGURE 11

FIGURE 12

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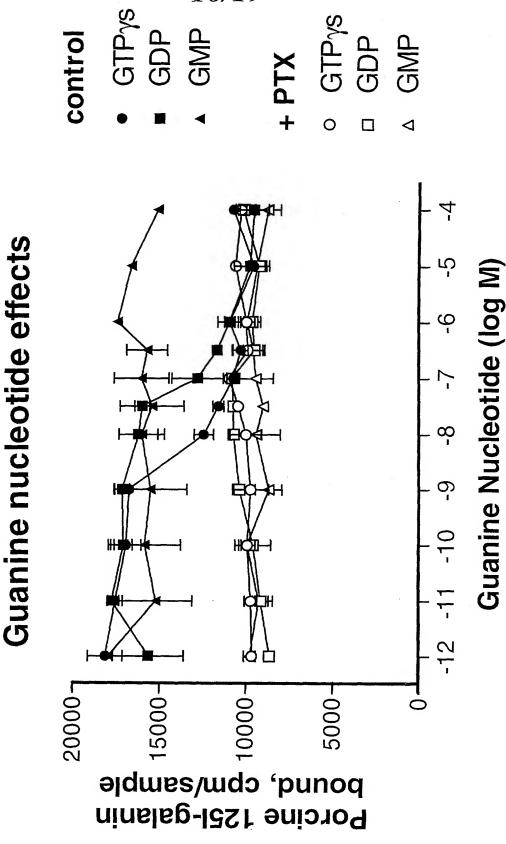Sheet 18 of 19

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FIGURE 13A

hGALR3-LM #228
+/- Pertussis Toxin

Guanine nucleotide effects

control
● GTPγS
■ GDP
▲ GMP

+ PTX
○ GTPγS
□ GDP
△ GMP

FIGURE 13B

Nonspecific Binding with 1 μ M p galanin